JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1975, p. 112-113

Vol. 1, No. 1
Printed in U.S.A.

Isolation of Infectious Bovine Rhinotracheitis Virus from Mustelidae

DAVID D. PORTER,* AUSTIN E. LARSEN, AND NANCY A. COX

Department of Pathology, University of California School of Medicine, Los Angeles, California 90024, and Department of Microbiology, University of Utah College of Medicine, Salt Lake City, Utah 84112*

Received for publication 20 September 1974

Herpesviruses isolated from either domesticated or wild carnivores should be immunologically compared with known viruses of this group which could have been included in the diet of the animal before being considered to be previously undescribed herpesviruses native to the carnivore in question.

Herpesviruses are widespread in nature and infect a variety of mammalians and lower vertebrates. The majority of these viruses have a narrow host range, and some are entirely species specific (4, 6). We report here the isolation of bovine herpesvirus 1 or infectious bovine rhinotracheitis (IBR) virus from four mink (*Mustela vison*) and one ferret (*Mustela furo*).

The first isolate of IBR was from a clinically normal young adult ferret. Trypsinized spleen, kidney, and lung were grown in 450-ml prescription bottles. Three days after plating the cells, foci of degeneration were noted in three of eight bottles of spleen cells but in none of eight bottles each of lung or kidney cells. The cell degeneration quickly spread to involve the entire cell sheet. None of about 50 other bottles of IBR-susceptible cells on which the same culture medium and trypsin solution were used developed a cytopathic effect, suggesting that it was unlikely that the viral isolate represented a contaminant in the fetal calf serum or trypsin.

The second isolate of IBR was from the homogenized intestine of a mink with clinically typical mink viral enteritis. When the homogenate was placed upon monolayers of secondary ferret kidney cells (nonpermissive for mink viral enteritis virus), foci of degeneration developed in 2 days and rapidly involved the cell sheet. The agent was reisolated from the homogenized intestine six times with an average titer of $10^{3.3}$ plaque-forming units (PFU)/g. Electron microscopic observation of cell cultures infected with the first and second isolates showed viral particles with a typical morphological appearance of herpesviruses.

Secondary ferret kidney cell cultures were found to support the growth of these isolates. An average titer of 10^6 PFU/ml was observed at the time of complete cell degeneration. All subsequent studies used these cells.

The isolates were plaque-purified using agarose overlays three times. Virus stocks were prepared in ferret kidney cells using serum-free medium. Antisera were prepared in rabbits by inoculating $10^{6.3}$ PFU of the mink and ferret virus isolates in complete Freund adjuvant three times at 3-week intervals. Serum neutralization tests were performed by mixing equal volumes of virus stock containing 200 PFU/0.2 ml with twofold dilutions of heat-inactivated serum, starting with a serum dilution of 1:10. All dilutions were made in tissue culture medium. After a 1-h incubation at room temperature, residual infectivity was determined by inoculating 0.2 ml of each mixture on triplicate secondary ferret kidney cell cultures in 60-mm plastic petri dishes. After a 1-h adsorption period, the monolayers were washed once with medium. A liquid overlay medium containing 2% fetal bovine serum and 3% heat-inactivated rabbit antiserum to the homologous virus was used to prevent the formation of secondary plaques (2). After 5 days of incubation at 37 C, the overlay medium was poured off, the monolayer was stained with 0.1% crystal violet, and the plaques were enumerated. Serum neutralization titers were expressed as the final serum dilution producing 80% plaque reduction.

Indirect immunofluorescence tests using acetone-fixed infected ferret kidney cells showed no reaction of the isolates with antisera to human cytomegalovirus AD169, monkey cytomegalovirus GR2757, varicella-zoster virus, EB virus, equine herpesviruses (types 1 and 2), feline herpesvirus C27, herpes tamarinus virus, pseudorabies virus, or Marek's disease virus. A weak cross-immunofluorescence reaction was found between human herpes simplex type one and

the mink and ferret isolates, but no cross-neutralization was noted. The Cooper strain of IBR virus and a rabbit antiserum to the Cooper strain of IBR virus were obtained from Jack G. Stevens (5). An extensive cross-immunofluorescence reaction was found between IBR virus and the mink and ferret isolates using antisera to the three viruses. The results of cross-neutralization tests are shown in Table 1 and indicate that the mink and ferret isolates are closely related to, if not identical to, the Cooper strain of IBR virus, whereas no reaction with other herpesvirus antisera was observed.

Three subsequent isolations of IBR virus have been made from primary spleen cell cultures of adult mink. These isolates were also found to be indistinguishable from IBR using the serological tests described above. As before, no evidence of IBR virus growth in other IBR virus-susceptible cells was noted when using the same bottles of medium and trypsin. Individual sera from four of the five animals from which IBR virus was isolated were available for serological testing. One serum showed a weak reaction with IBR by immunofluorescence, but none of the sera had detectable neutralizing antibody. This finding suggests that infection of the mink and ferret by IBR occurred only a short time before the animals were sacrificed. Large pools of mink and ferret sera (50 to 200 animals/pool), obtained from the same ranch as the animals from which IBR virus was isolated, were tested for IBR antibody using immunofluorescence and neutralization tests. No IBR antibody was detected in these serum pools. The incidence of IBR isolation from mustelid tissue is low. IBR was isolated from one of 41 sets of primary ferret cultures and from four of about 250 mink tissue suspensions or cell cultures.

TABLE 1. *Neutralization test using herpesviruses isolated from a mink and a ferret and IBR virus and antisera prepared to a number of herpesviruses*[a]

Antiserum to:	Mink isolate	Ferret isolate	IBR, Cooper strain
Mink isolate	48	48	95
Preimmune serum	<10	<10	<10
Ferret isolate	26	29	78
Preimmune serum	<10	<10	<10
IBR, Cooper strain	69	116	25
Preimmune serum	<10	<10	<10
Herpes simple type 1	<10	<10	<10
Cytomegalovirus AD169	<10	<10	<10
Cytomegalovirus GR2757	<10	<10	<10
Feline herpesvirus C27	<10	<10	<10
Equine herpesvirus 1	<10	<10	<10
Equine herpesvirus 2	<10	<10	<10
Pseudorabies virus	<10	<10	<10
Herpes tamarinus virus	<10	<10	<10
Varicella-zoster virus	<10	<10	<10
Marek's disease virus	<10	<10	<10
EB virus	<10	<10	<10

[a] Results are presented as the reciprocal of the serum dilution producing an 80% reduction in plaque count.

Eight mink were inoculated intraperitoneally with 10^6 PFU of the first mink isolate of IBR virus. None developed a clinical illness, but all had developed antibody when tested 3 weeks after viral inoculation. This does not prove that mink can be infected with IBR virus or that the virus replicated in the inoculated mink.

The diet of mustelids raised on commercial ranches in Utah where these animals were obtained contains 5% uncooked beef tripe (3). Other raw beef by-products are occasionally included in the diet if available in sufficiently large quantities at a low price. It seems highly likely that the original source of the IBR virus which we isolated from the tissues of these animals was the raw beef tripe or other beef by-products in the diet. The manner in which the virus got into the spleens of four of the five animals is not known. The diet of the wild carnivores can be simply stated as "what they can get" (1). We suggest that herpesviruses isolated from either domesticated or wild carnivores be immunologically compared with known viruses of this group which could have been included in the diet of the animal before being considered to be previously undescribed herpesviruses native to the carnivore in question.

This work was supported by Public Health Service grant AI-09476 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Ewer, R. F. 1973. Food and food finding, p. 139-229. *In* The carnivores. Cornell University Press, Ithaca, N.Y.
2. Hoggan, M. D., and B. Roizman. 1959. The isolation and properties of a variant of herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. Amer. J. Hyg. **70**:208-219.
3. Larsen, A. E., D. D. Porter, and H. G. Porter. 1971. Pregnancy-associated serum protein in mink. Proc. Soc. Exp. Biol. Med. **136**:430-432.
4. McKercher, D. G. 1973. Viruses of other vertebrates, p. 427-493. *In* A. S. Kaplan (ed.), The herpesviruses. Academic Press Inc., New York.
5. Stevens, J. G., and N. B. Groman. 1963. Properties of infectious bovine rhinotracheitis virus in a quantitated virus-cell system. Amer. J. Vet. Res. **24**:1158-1163.
6. Wolf, K. 1973. Herpesviruses of lower vertebrates, p. 495-520. *In* A. S. Kaplan (ed.), The herpesviruses, Academic Press Inc., New York.